Dkt. No. 96700/845

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Ekaterina Dadachova, Joshua D. Nosanchuk, and Arturo Casadevall
Appl. No.	:	10/775,869
Filed	:	February 10, 2004
For	:	RADIOLABELED ANTIBODIES FOR TREATMENT OF TUMORS
Group Art	:	1642
Examiner	:	Brandon J. Fetterolf
Customer No.	:	1912

DECLARATION OF EKATERINA DADACHOVA UNDER 37 C.F.R. §1.132

Commissioner for Patents
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Sir:

I, Ekaterina Dadachova, hereby declare as follows:

1. I am a co-inventor of the subject matter claimed in U.S. Patent Application No. 10/775,869. I am currently an Associate Professor in the Department of Nuclear Medicine and the Department of Microbiology & Immunology at Albert Einstein College of Medicine of Yeshiva University, Bronx, New York.

2. Described herein are additional data in support of the present invention, which were obtained with the anti-melanin monoclonal antibody 11B11 labeled with 188-Rhenium. Experiments were conducted using MNT1 pigmented human melanoma cells, which are described in the present application.

Monoclonal antibody (mAb) 11B11 was generated by immunizing BALB/c mice with purified *Cryptococcus neoformans* melanin followed by fusion of splenocytes to myeloma cells (Rosas AL, Nosanchuk JD, Feldmesser M, Cox GM, McDade HC, Casadevall A. Synthesis of polymerized melanin by *Cryptococcus neoformans* in infected rodents. *Infect. Immun.* 68(5):2845-53, 2000). The purified antibody was obtained from supernatant made by growing the 11B11 hybridoma cells in standard DMEM with 5% FCS. The antibody was captured on a column using agarose beads with anti-mouse IgM (Sigma) and eluted using acid then neutralized (pH 7). The antibody concentration was determined by ELISA by comparison to a commercial standard.

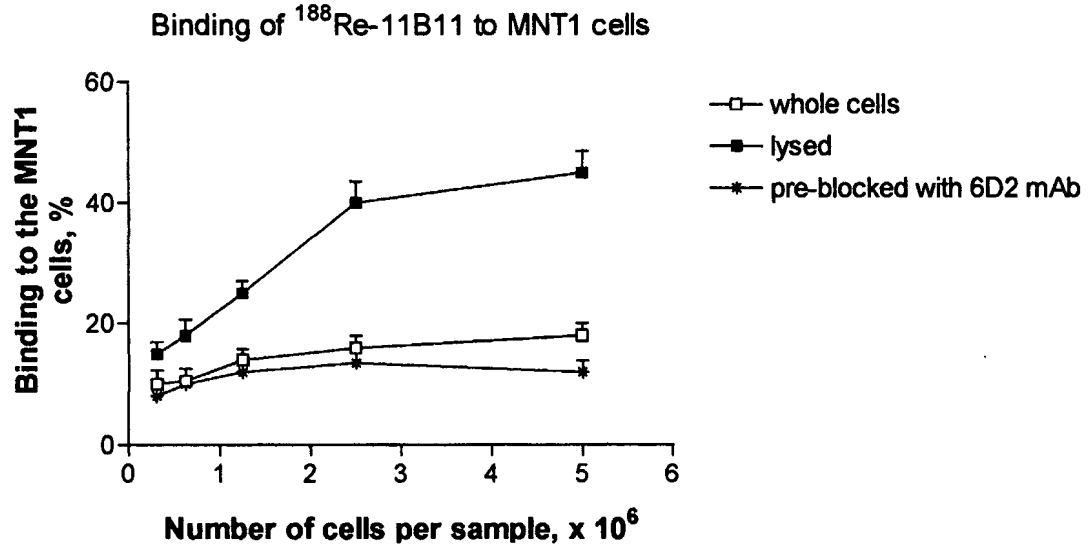
For the binding experiments ^{188}Re-11B11 was added in the amount of 0.106 nM to the increasing concentrations of whole MNT1 cells, whole MNT1 cells pre-blocked with the excess of 6D2 mAb or to osmotically lysed MNT1 cells in the centrifuge tubes pre-blocked with 1% BSA to prevent non-specific protein binding. After 1 hour incubation at 37°C the activity in the tubes was counted in a gamma counter, the cells were collected by centrifugation and the pellets were counted.

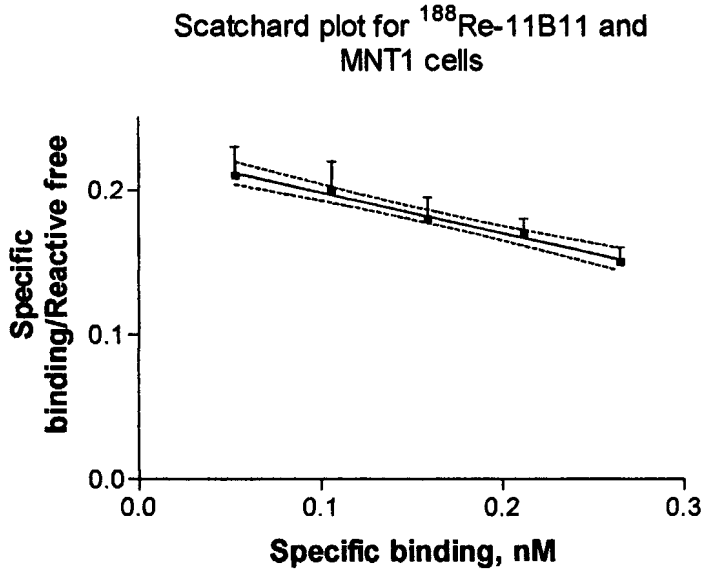
For Scatchard analysis of binding ^{188}Re-11B11 was added in increasing amounts (0.053 nM to 0.256 nM) to osmotically lysed MNT1 cells (4 x 10^6 cells per centrifuge tube pre-blocked with 1% BSA to prevent non-specific protein binding). After 1 hour incubation at 37°C the activity in the tubes was counted in a gamma counter, the cells were collected by centrifugation and the pellets were counted. Scatchard analysis was used to compute the mAb association equilibrium constant K_a as in Lindmo T, Boven E, Cuttitta F, *et al.* (Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J. Immunol. Methods* 72:77-89, 1984).

The results are illustrated in the accompanying figures. Binding of ^{188}Re-11B11 to MNT1 highly melanized cells was melanin-specific as lysing of the cells which makes

more melanin accessible for a melanin-binding mAb resulted in increased binding. 11B11 and 6D2 mAbs bind to predominantly different epitopes on melanin as pre-blocking of the cells with 6D2 had only relatively minor effect on the binding of ^{188}Re-11B11. Affinity constants for melanin-binding for 11B11 and 6D2 are 2.8×10^8 M^{-1} and 1.8×10^8 M^{-1}, respectively, which are close to each other.

Binding of ^{188}Re-11B11 mAb to MNT1 melanoma cells





Ka=2.8 x 10^8 M^{-1}; 1.2 x 10^5 binding sites per melanin are released from a single cell.

3. I hereby declare that all statements made herein and of my knowledge are true and that all statements made on information and belief are believed to be true; and I further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 4 April 2007

Ekaterina Dadachova